Lead in Bone. IV. Distribution of Lead in the Human Skeleton

LORENTZ E. WITTMERS, JR., Ph.D.
Department of Physiology
University of Minnesota-Duluth
School of Medicine
Duluth, Minnesota
JoANN WALLGREN, M.E.
Department of Pathology and Laboratory
  Medicine
University of Minnesota-Duluth
School of Medicine
Duluth, Minnesota
AGNES ALICH, Ph.D.
Department of Chemistry
College of St. Scholastica
Duluth, Minnesota

ARTHUR C. AUFDERHEIDE, M.D.
Department of Pathology and
  Laboratory Medicine
School of Medicine
Archaeometry Laboratory
University of Minnesota-Duluth
Duluth, Minnesota
  and
Center for Ancient Studies
University of Minnesota-Minneapolis
Minneapolis, Minnesota
GEORGE RAPP, JR., Ph.D.
Archaeometry Laboratory
University of Minnesota-Duluth
Duluth, Minnesota
  and
Center for Ancient Studies
University of Minnesota-Minneapolis
Minneapolis, Minnesota

ABSTRACT. Flameless atomic absorption spectroscopy was used to measure lead concentrations in samples from 5 selected human skeletal sites (tibia, skull, rib, ilium, and vertebra) obtained from 134 hospital autopsies. Lead was distributed unequally among the different bones in distinct patterns that were age-, and to some extent, sex-dependent. To estimate lead concentration of the entire skeleton, all skeletal bones were divided into 5 groups based on their approximate compact/trabecular bone ratios, considering each of our 5 sampled sites to be the prototype for each such group. Regression analysis of the 10 possible bone site pair values at different ages yielded age-related constants. These constants were incorporated into an equation we developed that can be used both to estimate mean skeletal lead concentration ($\overline{Pb}$) of the entire body skeleton and also to predict the lead concentration at any of the other 4 bone sites if any 1 of the 5 is measured. Applications of these data to in vivo bone lead measurements are detailed with respect to selection of the site to be measured, estimation of total skeletal lead burden, anticipated variations or error, and dependence of these factors on age and sex of the sampled population.

CENTURIES before the earliest written records, lead was a widely used metal, and it remains so today. Human exposure to this element can result in serious pathological consequences if the body content reaches a critical level. In humans, blood is the most common tissue sampled for lead analysis, and the medical literature relates the clinical features of lead toxicity to these blood lead levels.

The majority of human lead uptake occurs via the respiratory and gastrointestinal tracts. As much as 40% of the inhaled lead is absorbed from the lung[1] and enters the circulatory system. Gastrointestinal absorp-

tion of this metal is age-dependent; adults absorb about 10% of lead ingested, whereas in children this fraction may reach as high as 50%.[3] Lead can be absorbed directly through the skin, but this route is insignificant unless the concentration is high and contact time prolonged. Ninety percent of the lead leaves the body via the urine, and most of the remainder is excreted with the feces; only a very small amount is lost in sweat, hair, and nails.[4]

Lead is distributed unequally throughout the tissues of the body. Less than 10% of all lead stored in the body is deposited in the soft tissues, but the skeleton contains the remaining 90-95%.[1] In bone, lead is incorporated into the hydroxyapatite crystal from which it can be mobilized only very slowly. Recently administered lead, however, seems to be more easily mobilized.[5]

Studying lead turnover kinetics, Rabinowitz et al.[5] carried out lead balance studies by supplementing the constant hospital diet of five volunteers with nonradioactive lead. Employing a three-compartment model, they predicted mean lead half-lives of 36, 40, and 10⁴ days, respectively. Using these data, Batschelet et al.[6] expanded the model to include the lungs and gastrointestinal tract. This refinement of the model yielded average half-lives of 15.5, 34.7, and 22.6×10^3 days for blood, soft tissues, and bone, respectively. Smith and Hursh,[1] using values published by various workers, computed lead half-lives for blood, liver, and bone of 69, 650, and 4,250 days. It is clear that bone-lead residence time (at least in the adult) is of a magnitude to justify an attempt to use adult bone lead content as a reasonable reflection of lifetime lead exposure.

Distribution of lead in the human skeleton. For many years the laboratory diagnosis of lead poisoning has been achieved by quantitation of lead in blood and urine samples.[7-10] Skeletal lead content has been of interest primarily to physiologists, epidemiologists, and paleopathologists dealing with the question of prolonged lead exposure, in many cases attempting to estimate total lifetime lead accumulation.[11-13] Recent concern for chronic and subclinical lead intoxication, often acquired during occupational exposure, has broadened interest in bone lead levels.[14,15] These interests have resulted in the development of in vivo lead quantitation either by bone needle biopsy[16] or by noninvasive x-ray fluorescence techniques.[17]

Because earlier work has demonstrated inhomogeneity of skeletal lead distribution,[11,18] selection of the bone site for noninvasive and/or single-site sampling techniques becomes critical. In addition, application of skeletal lead analysis to archaeological bones has generated a similar need because often only limited bone sample sites (not always the same ones) are available for analysis. For appropriate interpretation, the relationship of the lead concentrations at the available site to that of the total skeletal lead level must be known.

The information presented here is the creation and analysis of a database designed to address the above noted specific concerns, with particular reference to the following questions: (1) How is lead distributed among various bones of the human skeleton in modern industrial populations? (2) How do the lead concentration patterns of the various bones of the human skeleton differ in relation to age and sex? and (3) Can a method be developed for prediction of total body skeletal lead concentration and burden that would be a more consistent standard for comparison than measurement of any one bone site?

Materials and methods

Sample sites. Between 1976 and 1982, bone samples were obtained by a single pathologist from 134 random, northern Minnesota, community hospital autopsies. This population included 81 Caucasian males and 53 females ranging in age from 0 to 98 yr (Fig. 1). The bone sample sites were as follows: (1) tibia (midshaft); (2) vertebra (wedges from the bodies of the third and fourth lumbar and the fifth thoracic vertebrae measuring 3 cm along the edge of each face). Only the four lumbar site was sampled after enough data had been analyzed from the other vertebral sites to demonstrate no significant lead content differences among them (see Results); (3) rib (a segment 6 to 9 cm lateral to the costochondral junction of the left fourth rib); (4) ilium (a full thickness rectangular block 5 × 5 cm, one edge of which included the iliac crest, removed from the right ilium approximately 5 cm posterior to the anterior superior spine); and (5) skull (upper left occipital bone). All samples were full-thickness sections, each wrapped in plastic and frozen until time of preparation for lead analysis. For analysis, 3-mm diameter samples were acquired using an electrically driven, stainless steel, hollow core bit, either directly from the cadaver (tibia) or from the larger stored samples.

Lead analysis. A detailed description and validation of this method for bone lead analysis has been presented elsewhere.[19] Therefore, only a summary will be presented here.
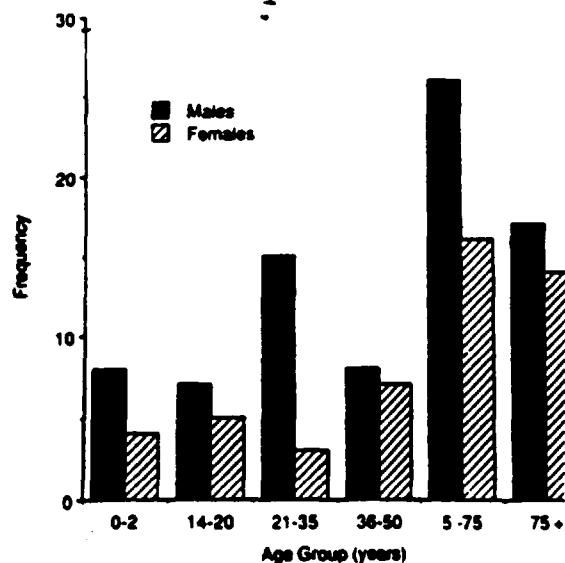

Fig. 1. Characterization of the entire study population, as a function of age and sex. Note: our study population had no subject in the 3-13 yr age range. Total number of males = 81; total number females = 53.

The bones were thawed and scraped to remove adhering soft tissue. Samples were placed in Vycore crucibles and dried at 110°C for 20 hr (to constant weight). The samples were ashed in a muffle furnace at 450°C for 3 hr (or until completely white), and cooled in a desiccator. The ashing temperature was selected to avoid loss of lead by volatilization or as the sulfide or chloride. The ashed weight was recorded, and the samples were ground to a fine powder in an agate mortar; samples were returned to the desiccator until analysis.

Bone ash was dissolved in nitric acid, diluted, and an aliquot was transferred to a sampling cup and diluted again with 1.0 ml of water containing 853 μg/ml lanthanum ion. Analysis was accomplished with flameless atomic absorption spectroscopy. Two standard bone samples, one high and the other low in lead levels, were included in the analysis as quality control samples for each day's run. These standard samples were bulk bone ash that were stored in a desiccator and redried at intervals. They were treated in the same manner described for the samples.

To minimize contamination, all glassware, crucibles, and sample cups to contact the samples and standards were soaked in nitric acid (HNO₃) (7.8 moles/L) from 2 to 4 hr during the cleaning process.

The samples were analyzed on a Perkin-Elmer HGA 2100 graphite furnace, which was programmed as follows: drying time 30 sec, temperature 100°C; charring time 30 sec, temperature 475°C; atomizing time 7 sec, temperature 475°C; wavelength 283.3 nm; integration time 6 sec; background correction—on; sample size 20 μl.

Equation [1] below was developed to calculate mean lead concentration of the total body skeleton (Pb) using values actually measured at all five sites (see Appendix for detailed derivation).

$$(Pb) = W_s/W_a[(Pb)_1R_1W'_a/W'_1 \ldots + (Pb)_sR_sW'_a/W'_s] \quad [1]$$

Certain additional methodological aspects are discussed in direct reference to their application under Results.

Results

In the development of the method, multiple measurements of the low standard bone yielded a coefficient of variation (CV = SD × 100/mean) of 12.4%, and for the high standard bone the coefficient of variation was 8.6%. The recoveries were not a function of bone lead concentration and were 103% ± 12.9% (SDM). The absolute sensitivity of our method was 70.6 ± 10.8 × 10⁻¹² g lead. A 0.006 μg lead/ml solution yielded a detection limit of 0.0021 μg·lead/ml, and a 0.015 μg lead/ml solution yielded a detection limit of 0.0065 μg lead/ml.[19]

Concentration units. Various laboratories have reported bone lead concentrations as the amount of lead per gram of wet bone, dry bone, or bone ash.[18,22,23] We have elected to express our data as "micrograms of lead per gram bone ash" in consideration of the errors inherent in obtaining accurate wet and dry weights. However, to facilitate the comparison of our data with that

published as wet and dry values we measured the wet, dry, and ashed weights on 30 adult samples in our study population. These ratios are summarized in Table 1.

To evaluate age effects on the change in weight conversions from ash to dry or wet values, the ash to dry and ash to wet ratios for each bone site were plotted as a function of age. The data were fit by linear regression and yielded slopes of between 10⁻³ and 10⁻⁴ with regression coefficients ranging from .07 to 0.6. Due to the very small slopes and the large data scatter, we did not attempt corrections for weight ratio differences as a function of age.

Lead distribution

Bone site differences. Table 2 and Figure 2 present the lead concentration, as a function of age, for bones at the 5 sample sites studied. The age groupings were selected on the basis of developmental and physiological function: 0–2 yr, infants; 3–12 yr, children (in the data presented we had no individuals in this group): 13–20 yr, adolescents; 21–35 yr, young adults; 36–50 yr, mid-adults; 51–75 yr, mature adults; and >75 yr, senior adults. Table 3 summarizes the results of nonparametric analysis (Wilcoxon Signed Rank Test[4]) of the 10 possible bone pair comparisons among the 5 sample site values.

The newborn-infant group is omitted from this and subsequent evaluations for the following two reasons: (1) there are too few subjects in this group for proper statistical analysis, and (2) the lead concentration of all samples is below the detection limits of the method. In adolescent and young adult groups, only the vertebral values differ significantly from the other four sample sites. Differentiation occurs between other bone pairs in the 36–50 yr age group. Following that age, the concentration at each bone site is statistically different from that at every other site ($p < .05$).

Linear distribution of lead in the diaphysis of the tibia. Sample site selection in any long bone would become a significant variable if there are substantial differences in lead concentation at various points along the diaphysis. For this reason, bone lead concentrations were measured at many sites along the diaphyseal length of the tibia. This bone was an intact right tibia from a 56-yr-old male logger's skeletonized, exposed body found in the forest 18 mo after his disappearance. Beginning at the proximal end, 3-mm core samples (full-thickness) of the bone cortex were removed at 1-cm intervals along the length of the diaphysis (a total of 28 samples).

Analytical values are demonstrated in Figure 3. Least squares regression analysis[24] was applied to the lead concentration of the individual samples (dots in Fig. 3) as a function of distance along the diaphysis. The slope of this regression line was −0.06 with a regression coefficient (R^2) of 0.01. This analysis indicates that there is no linear relationship between bone lead concentration and the distance (position) along the diaphysis. In vivo monitoring systems commonly scan bone surface areas of about 3-cm diameter.[15,25] To approximate such conditions more closely, we averaged the above de-

Sample site	Dry/weight ± SEM	Ash/wet ± SEM	Ash dry ± SEM	N
Tibia	0.878 ± 0.005	0.531 ± 0.004	0.605 ± 0.012	16
Vertebra	0.576 ± 0.013	0.174 ± 0.011	0.301 ± 0.015	37
Rib	0.618 ± 0.014	0.224 ± 0.012	0.372 ± 0.018	33
Ilium	0.695 ± 0.010	0.287 ± 0.012	0.411 ± 0.014	23
Skull	0.844 ± 0.010	0.533 ± 0.012	0.632 ± 0.010	14

Table 2.—Mean Bone Lead Concentrations (µg Pb/g bone ash) as a Function of Age for the Five Sites Sampled

Age group (yr)	Age (yr)	Tibia	Ilium	Rib	Vertebra	Skull
>75	86.3(31)*	29.0(28)	17.0(29)	20.5(31)	18.8(30)	26.1(28)
SEM	±1.0	±3.4	±2.6	±2.4	±2.6	±3.2
51-75	63.9(42)	24.2(38)	19.2(40)	22.3(40)	22.4(41)	22.8(29)
SEM	1.1	2.3	2.4	2.6	2.6	2.9
36-50	42.3(15)	16.6(14)	9.9(15)	9.7(15)	11.9(15)	15.2(15)
SEM	1.3	4.1	1.6	1.7	2.1	3.3
21-35	24.6(18)	5.9(18)	5.3(16)	5.0(18)	6.3(17)	4.9(17)
SEM	1.0	1.2	1.2	1.2	1.3	1.1
14-20	17.6(13)	2.3(13)	2.3(13)	2.9(12)	3.8(12)	3.2(10)
SEM	0.5	1.0	0.9	1.4	1.4	1.7
0-2†	0.3(12)	0.3(11)	0.0(11)	0.7(12)	0.6(12)	0.6(12)
SEM	0.1	0.2	0.0	0.4	0.6	0.4

Note: our sample population contains no subjects between the ages of 3 and 13 yr.
*Numbers in parentheses represent the total number of samples contributing to the mean value.
†The 0-2 yr age group is included here to emphasize the low lead levels. It is not considered in subsequent tables (see text for discussion).

scribed core sample values in sequential groups of three along the entire diaphyseal length, thus obtaining the mean values over 3-cm increments. These 3-cm grouped samples are presented as bars in Figure 3. The overall mean and standard deviations of such groupings were 28.5 ± 4.1 µg Pb/g ash. All the 3-cm groupings fall within ± 1 standard deviation of the overall mean value.

We concluded that for the employment of in vivo bone-lead measuring devices, differences in bone lead concentration along the length of the tibial diaphysis are small enough so they may be neglected.

Lateral asymmetry. Skeletal morphological asymmetry is well known,[3] requiring demonstration that lateral dominance does not affect bone lead concentration. Values for lead content of samples from both left and right tibial diaphyses of 12 adult members of an archeaologically excavated colonial American population[13] were determined and are displayed in Table 4. Analysis of this data employing the paired Student's t test[24] yielded a t statistic of 0.18 with 11 degrees of freedom, indicating no significant difference between right and left sampling sites of the same individual.

Vertical asymmetry. Because weight-bearing stress may vary at different vertebral levels, we obtained samples from two adjacent lumbar vertebrae (L_3 and L_4), and from one thoracic vertebra (T_3) in 22 autopsies. Their bone lead concentrations were treated with one-

way analysis of variance and yielded no significant difference ($p < .05$) among any of the three possible sample-site pairs (L_3 vs. L_4, L_3 vs. T_3, and L_4 vs. T_3). Thereafter, a mean vertebral lead level was assigned to all autopsies, in which more than one vertebra was sampled. In subsequent autopsies, vertebral samples were obtained only from the L_4 site. It was felt that the absence of difference among the three sites also reflected consistency of our sampling, storage, and analysis techniques.

Metaphysis vs. diaphysis. Because lead is deposited preferentially at sites of most active bone growth,[1] it is possible that such deposition in the periepiphyseal areas of the metaphyses of long bones during the year of body growth might result in lead concentrations that are different from those in the diaphysis.

Samples were prepared and analyzed from the following sites in adult tibias: mid-diaphysis cortical bone, metaphyseal cortical bone, and metaphyseal trabecular bone (metaphyseal samples both were acquired from just above the tibial tuberosity) in 47 autopsies. This population consisted of 31 males and 17 females with comparable mean ages (males 64.2 ± 3.0 SEM yr and females 68.7 ± 3.1 SEM yr). Meticulous care was used in the separation of all trabecular bone from the cortical samples. Student's t tests were applied to the three sets of data and are summarized in Table 5. No significant difference was found in the lead concentr.

...on of the cortical bone samples taken from the ...etaphysis and diaphysis of the same individual, but ...the lead concentration of the trabecular bone in the ...etaphysis was significantly different (usually higher) ...om that of either cortical sample ($p < .05$).

Lead concentration related to age and sex. Figure 4 demonstrates the pattern of lead concentration for the ...e sample sites in relation to each age group. The ...rgely compact bone sites (tibia and skull) reveal a ...erpetually rising lead concentration, whereas those ...ontaining a significant component of trabecular bone ...cline in the oldest age group. The degree of this ...cline is roughly proportional to their fraction of ...rabecular bone content.

To define these relationships further, the values of ...he purely compact bone site sampled (tibia) were plotted ...ith those of the purely trabecular bone site (vertebral

body) for both males and females (Fig 5) It is noted that vertebral levels exceeded those of the tibia until the growth period was completed at about the age of 20 yr. However, after age 35 yr, tibial levels were uniformly greater. The largest discrepancy between these two becomes apparent in the oldest age group secondary to the marked decline in the vertebral lead concentration. The plotted tibia/vertebra ratio (Fig. 6) demonstrates a progressive discrimination in the adult population against lead deposition in the trabecular bone (vertebra) in relation to that in the cortical bone (tibia). Similar patterns are evident in the other two primarily trabecular bone sites sampled (rib and ilium). Tibial/ skull ratios are interesting in that the skull parallels vertebral values during youth and tibial values during adulthood. This may well reflect the small diploic component of the adult occipital bone. These relationships may be more easily visualized in Figure 2, which summarizes the mean lead concentrations at each of the five bone sites for each of the age groups.

Estimation of total body skeletal lead concentration. If the total pool of lead stored in the human skeleton can ultimately be mobilized and therefore constitutes a threat of latent toxicity to its host, it would be desirable to estimate the magnitude of the total body lead burden and its location in the principal compartments, compact and trabecular bone. Our selected sample sites represent a gradient of compact/trabecular bone ratios from pure compact bone (tibia) through mixtures of compact and trabecular bone of varying degree (rib and ilium) to pure trabecular bone (vertebral body).

Based on the compact/trabecular bone ratio of each bone, the entire complement of bones in the skeleton was divided into five categories, each represented by one of the bone sites sampled in this study. It was assumed that the lead concentration of the sampled bone site represented that of all the bones assigned to that group. Such a grouping was based on the weight distribution values of Lowrance and Latimer[*] and is presented in Table 6.
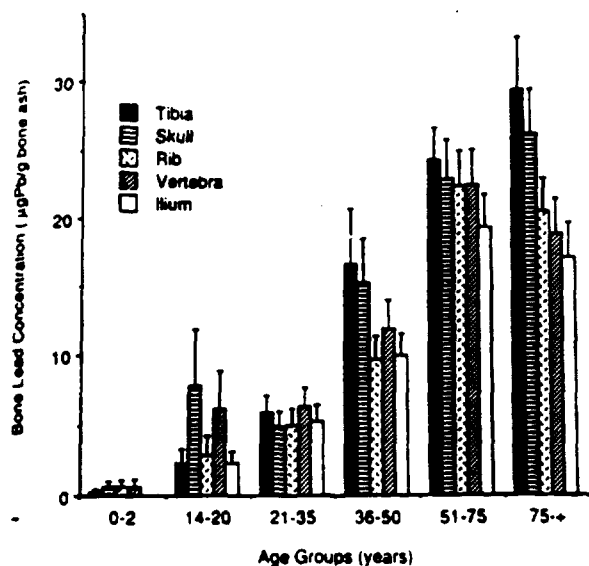
Fig. 2. Bone lead concentration at the five bone sites as a function of age group.

Y axis: Bone Lead Concentration (µgPb/g bone ash); 0, 10, 20, 30. Legend: Tibia, Skull, Rib, Vertebra, Ilium. X axis: Age Groups (years) — 0-2, 14-20, 21-35, 36-50, 51-75, 75-+.

Table 3.—Probabilities That Lead Bone Concentration Differences at the Indicated Sample Site and Age Are Due to Chance

Data pairs*	Age group (yr)				
	14-20	21-35	36-50	51-75	>75
T vs S	0.306 (7)	0.490 (16)	0.431 (13)	0.000 (26)	0.000 (26)
T vs R	0.390 (8)	0.326 (17)	0.021 (12)	0.000 (37)	0.000 (28)
T vs V	0.019 (9)	0.074 (16)	0.350 (13)	0.000 (38)	0.000 (28)
T vs I	0.288 (8)	0.431 (13)	0.011 (12)	0.000 (35)	0.000 (27)
S vs R	0.155 (7)	0.486 (13)	0.006 (14)	0.000 (27)	0.000 (27)
S vs V	0.031 (7)	0.015 (15)	0.099 (14)	0.000 (27)	0.000 (27)
S vs I	0.250 (5)	0.325 (13)	0.003 (13)	0.000 (28)	0.000 (26)
R vs V	0.014 (9)	0.012 (15)	0.037 (14)	0.004 (40)	0.000 (30)
R vs I	0.444 (8)	0.425 (14)	0.337 (13)	0.000 (36)	0.000 (28)
V vs I	0.006 (8)	0.006 (16)	0.048 (14)	0.000 (39)	0.000 (28)

Note: R = rib, T = tibia, V = vertebra, I = ilium, and S = skull.
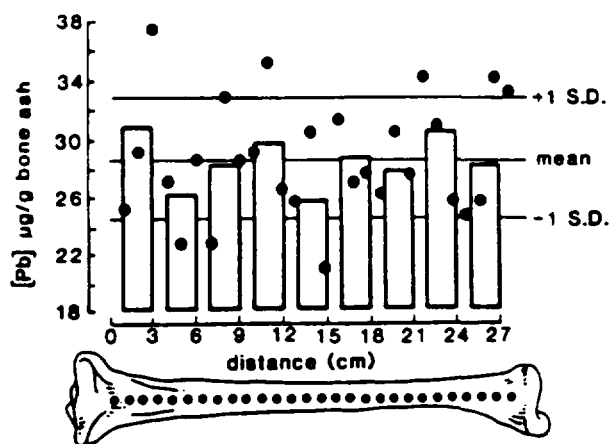*Numbers in parentheses represent number of paired analyses done in each group.

Fig. 3. Linear distribution of lead in the diaphysis of the tibia. The dots represent the individual sample lead concentrations (µg/g bone ash). The vertical bars represent the mean of three samples over a 3-cm distance. The mean and standard deviation values represent an analysis of all the samples taken from the tibia. See text for further discussion.

Table 4.—Lead Concentration (µg Pb/g bone ash) in the Right and Left Tibia of Archaeological Skeletons of 12 Colonial American Adults

Sample number	Lead concentration (µg Pb/g bone ash)	
	Right tibia	Left tibia
1	52.0	53.7
2	48.5	47.7
3	93.3	103.7
4	124.4	112.9
5	187.1	193.6
6	36.5	44.8
7	60.3	69.0
8	46.1	35.7
9	44.3	58.1
10	90.5	62.2
11	23.2	33.0
12	5.7	4.9

Discussion

As lead accumulates in the skeleton, evidence of differential distribution among the bones occurs. The earliest differences become apparent during adolescence (Table 3), when the trabecular bone of the vertebral body accumulates significantly more lead than that of the other four sites. The young adult period is characterized by further differentiation of lead concentrations in the bones studied until the fourth decade, after which the amount of lead stored at each of the five sites studied is uniquely different from that at the others.

The quantitative trends responsible for these changes can be identified in the age-related lead concentration patterns of the different sites. Until bone growth ceases at the end of the second decade, lead accumulates more rapidly in the trabecular bone sites, especially in the vertebral body (Fig. 2). During this phase of lead

storage the concentrations in these bones exceed those of predominantly compact cortical bone such the tibia. After body growth ceases, however, the verse becomes true. Lead is then preferentially stored in compact bone. This pattern continues through life, even beyond the eighth decade when the trabecular bones cease lead accumulation, yielding their previous lead content as reflected in their declining lead concentrations even while the compact bone of the tibia maintains its previous level (females) or continues to store ever greater quantities (males).

Varying selection of bone sample sites may be responsible for conflicting findings reported by different investigators regarding the rate of bone lead accumulation during the age period 40-90 yr. Some of these investigators reported their results in dry or wet bone units. Wherever necessary, we replotted such data after converting the lead concentrations originally reported in wet or dry units to ashed values. Using weight ratios listed in Table 1. The data reported by Gross et al.[14] and Drasch[8] yielded a pattern similar to that presented here in that, even after the age of 70 yr, the long bone cortical lead values continued to rise but those of the trabecular bones declined. Among those investigators who measured lead concentrations in bones with a major fraction of trabecular bone structure (usually rib samples), a decline in lead concentration in advancing age was noted by Cherry et al., Schroeder and Tipton,[11] and Nusbaum et al.,[10] but not by Barry[23] or Ulrich.[25] On the other hand, Weinig and Borner[31] found no such decline in either cortical or trabecular bone samples, although the latter two investigators had only two subjects over the age of 70 yr in their populations. It is conceivable that the more rapid lead turnover rate of the trabecular bone may be responsible for these patterns (see discussion below) augmented by the higher rate of lead absorption in children.

Because it is not feasible to measure the lead content of five different sites in vivo, it was hoped that the differences in lead concentrations of various bones would be sufficiently consistent in their relationships to each other that prediction of the values at four of the five sites would be possible after actual measurement of any one of them. Equation [1] could then be used to estimate the mean lead concentration of the entire skeleton. After that was achieved, the total skeleton lead burden could be expressed in absolute terms by multiplying its mean skeletal lead concentration (Pb) value by the weight of the entire skeleton (obtained from standard reference tables based on body weight).

To predict the lead content of 1 bone site from the analysis of another site, the database was sorted to yield only those subjects that had measured values for all 5 sites. The data were then separated into age groups as defined earlier. For each pair of bone sites (10 in all), the lead levels were fit with a straight line by the method of least squares.[24] The fitting procedure was done with the constraint that the intercept would be zero (0,0). The resulting slopes relating each of the bone pairs are summarized in Table 7. Employing the relationships summarized in Table 7, the bone lead

Table 5.—Comparison of Lead Content in Metaphysis and Diaphysis of the Tibia

	Metaphysis trabecular bone vs metaphysis compact bone	Metaphysis trabecular bone vs. diaphysis compact bone	Metaphysis compact bone vs diaphysis compact bone
Mean difference	7.24	8.06	0.82
Standard deviation	8.06	16.68	10.85
t statistic	2.97	3.31	0.53
p value*	<.005	<.005	.70

*Degrees of freedom for all comparisons was 46.
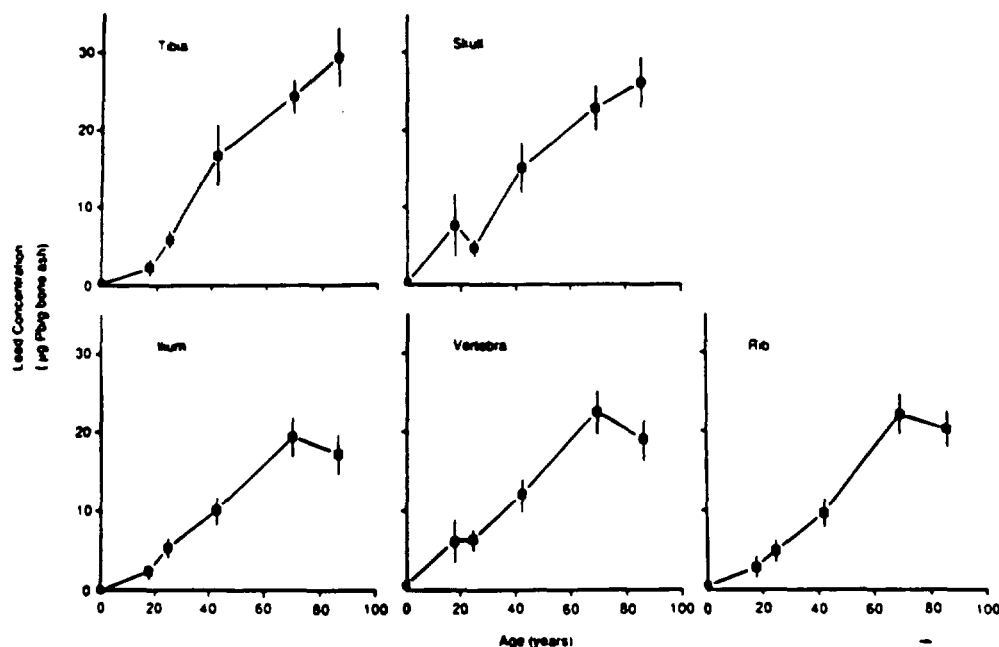
Fig. 4. Bone lead concentrations (µg/g bone ash) in relation to age for the five bone sites sampled.

concentration determined at only 1 site can be used to predict the lead concentrations in the bones at the other 4 sites.

Figure 7 demonstrates the variation of mean lead concentration of the entire skeleton estimated as follows: (A) actual measurement of the lead concentration at five different sites with the use of these values in Equation [1] (results indicated as A in Fig. 7); (B) actual measurement of the lead concentration at only one bone site (either tibia or vertebra), with mathematical prediction of the lead concentration at the other four sites and use of these values in Equation [1] ([B − A]/A × 100 is represented as B in Fig. 7); and (C) actual measurement of the lead concentration at only one site either tibia or vertebra), and assumption that the mean lead concentration of the entire skeleton is simply equal to that one measured value without any use of Equation [1] ([C − A]/A × 100 is represented as C in Fig. 7).

Values derived from measurements at only two of the single bone sites (tibia and vertebra) were plotted be-

cause values derived from rib and ilium single-site selection proved to demonstrate values similar to or intermediate between those of the vertebra and tibia, whereas the skull varied directly with the tibia in adults and with the vertebra in adolescents.

Perusal of Figure 7 reveals a progressive decrease in variations with increasing age. In addition, selection of compact bone as a single measured site produces approximations closest to the "actual" values for mean lead concentrations of the entire skeleton as estimated from actual measurements at all five sites ("A"). Predictions generated from measurements made only at a trabecular bone site, such as the vertebra, reveal much greater variations at all ages. Such differences between trabecular and compact bone certainly reflect that compact bone represents at least two-thirds of the skeleton's total weight and so carries a larger weighting constant in Equation [1]. In addition, some metabolic factor may affect trabecular more than cortical bone. If the presumably more sedate life activities of our oldest age group subjects resulted in decreased lead exposure
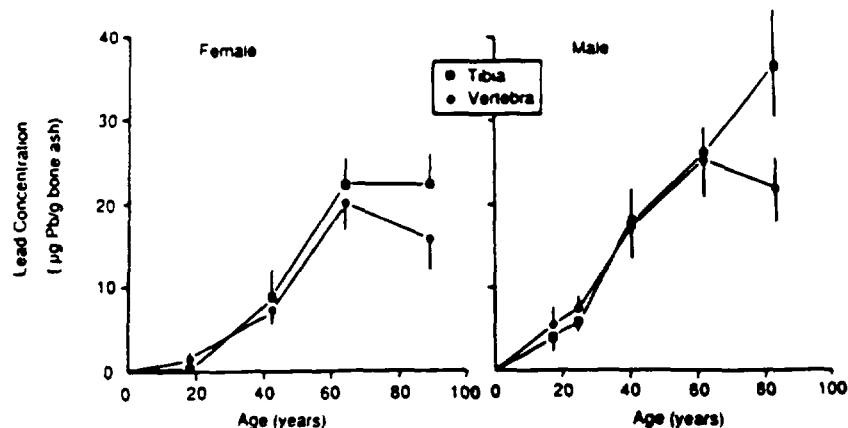
Fig. 5. Bone lead concentration (µg/g bone ash) in the tibia and vertebra as a function of age and sex.
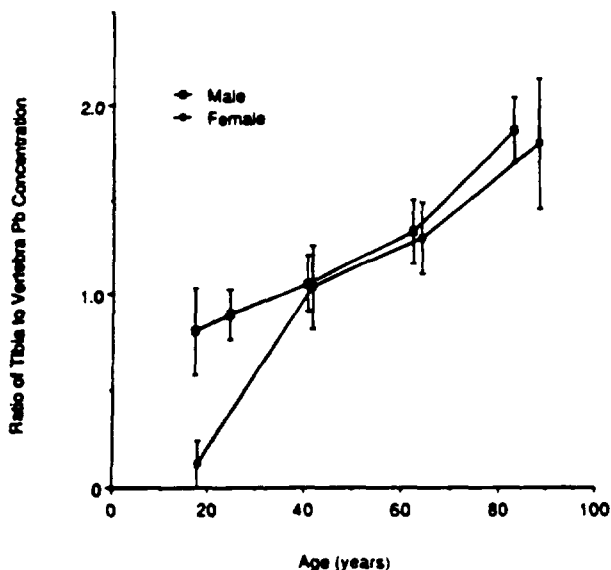
Fig. 6. Age-related tibia/vertebra ratios of bone lead concentration.

and absorption, then the more rapid turnover rate of trabecular bone[12] may be responsible for their observed decline in vertebral lead content. Furthermore, the absence of such a decline (or actual rise) of concurrent compact bone lead values may indicate that some of the lead that leached from trabecular bone was redeposited in compact bone. Finally, disease processes such as osteoporosis, common in the elderly, may affect trabecular bone selectively or predominantly. Such biological changes may contribute further to the mathematical impact consequent to the disproportionate representation of compact bone in the skeleton, resulting in greater variation in prediction of total skeletal lead burden when selecting trabecular rather than compact bone as the actual measured site.

These data have useful application in studies directed at the quantitation of human bone lead content for clinical use, whether by in vivo x-ray fluorescence

methods[33] or biopsy techniques.[34] Knowledge of th different patterns with respect to age that characteriz the various sites will assist the investigator in choosir the one most appropriate for the goal of a particul study. Interest in monitoring the total skeletal lea burden of industrially exposed workers would lead the selection of the tibia (or its equivalent) as a si whose concentration alone would most closely appro imate the desired measure. Modification of the simp tibia lead concentration using the appropriate pr diction constants would generate an even more a curate estimate in most age groups, still closer to tt "actual" mean skeletal lead concentration.

Table 6.—Grouping of Bones to Estimate Percentage of Total Skeleton Represented by Each Sample Site*

Bone	Percentage of total skeleton	Sample site
Tibia	10.63	
Humerus	6.38	
Radius	2.18	
Ulna	2.66	
Femur	17.67	
Hand	2.53	
Patella	0.57	
Fibula	2.47	
Foot	5.79	
Subtotal	50.88	Tibia
Skull	17.98	
Mandible	2.42	
Hyoid	—	
Subtotal	20.40	Skull
Rib	6.42	
Sternum	0.47	
Subtotal	6.89	Rib
Vertebra	10.06	Vertebrae
Ilium	7.83	
Scapula	2.84	
Clavicle	1.04	
Subtotal	11.71	Ilium

*Adapted from Lowrance and Latimer, Table 1 (1976).

Data pairs†	Age group (yr)				
	14-20	21-35	36-50	51-75	>75
S/T	1.247(.97)	0.947(.82)	0.919(.45)	0.989(.93)	0.815(.88)
R/T	1.198(.95)	1.034(.80)	0.695(.88)	0.951(.83)	0.632(.83)
V/T	1.315(.94)	1.112(.62)	0.837(.76)	0.906(.64)	0.557(.52)
I/T	0.883(.96)	0.928(.63)	0.684(.90)	0.816(.71)	0.652(.79)
S/R	1.030(.99)	0.839(.84)	1.262(.92)	1.000(.90)	1.234(.84)
S/V	0.950(.98)	0.689(.65)	0.989(.81)	0.939(.55)	1.261(.58)
S/I	1.361(.94)	0.830(.68)	1.297(.93)	1.060(.75)	1.346(.76)
V/R	1.071(.95)	1.093(.92)	1.144(.72)	0.941(.76)	0.893(.74)
I/R	0.699(.93)	0.903(.91)	0.966(.96)	0.870(.90)	0.928(.85)
I/V	0.639(.94)	0.815(.95)	0.737(.75)	0.851(.87)	0.946(.80)

Table 7.—Linear Regression Correlation* of Bone Sites as a Function of Age

Note: R = rib. T = tibia, V = vertebra, I = ilium. and S = skull.
*The correlation was accomplished with the equation $y = mx$: that is. the intercept was forced through (0.0).
†Numbers in parentheses represent correlation coefficient (R) for each of the sample site pairs.
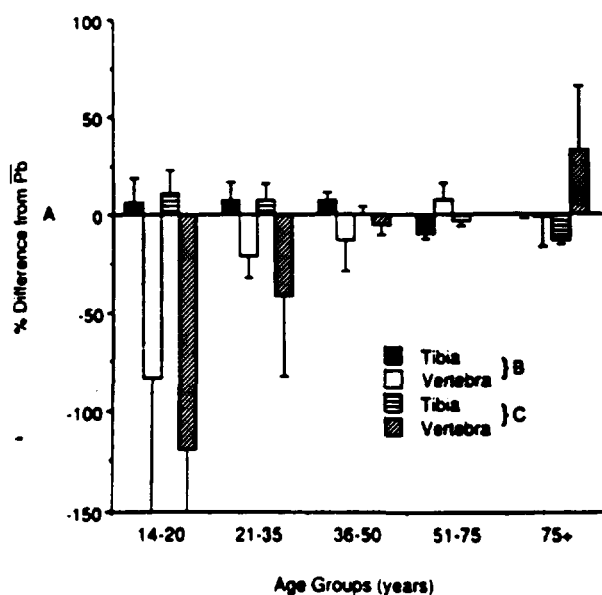
Fig. 7. Estimation of the accuracy of mean skeletal lead concentration of the entire skeleton using one or multiple bone sampling sites. A = mean lead concentration of entire skeleton estimated by actual measurement of five bone sites and using Equation 1 to calculate Pb. B = percent of A, represented by estimates of the mean lead concentration of the entire skeleton obtained by measurement of only one bone site (either tibia or vertebra), predicting the lead concentration at the other 4 sites and then using Equation 1. C = percent of A, represented by a value of mean lead concentration of the entire skeleton, assumed simply to be identical to actual value of only one measured bone site, either tibia or vertebra.

A study involving estimation of the trabecular bone lead content of the entire skeleton could use any of three such sites (vertebra, ilium, and rib) we measured or their equivalents. Our data indicate, however, that the lead concentrations at these sites are often not only substantially different from those of the compact bone

in the tibia, but frequently differ from those in other trabecular bone sites as a function of age (Fig. 2). Use of Table 7 prediction constants for these sites will contribute even more toward standardizing comparisons. Nevertheless, the data reveal that the investigator using such trabecular bone sites can expect a significantly greater variation in prediction of total skeletal lead burden than when using compact bone sites. This most likely reflects the impact of metabolic factors that are absent or operating to a lesser degree in the compact bone sites.

Bilateral sampling (Table 4) has assured us that either the right or left side may be employed with equal confidence, and no differences in lead concentration along various levels of the spine were found in the vertebrae analyses. Our data not only confirm the variably higher lead concentration of the long bone ends noted by previous investigators,[14,15] but also identify this increase as the exclusive contribution of trabecular bone content. Although minor inhomogeneities of lead deposition are demonstrable in the tibial diaphysis, these increase the variability of only the smallest samples: measurements of a 3-cm sample area (common in x-ray fluorescence in vivo techniques) anywhere along the length of the diaphysis will result in a value within one standard deviation of the mean value of the entire tibial shaft.

We anticipate that these data will be of substantial assistance in the design of human skeletal lead studies and in the interpretation of the resulting analytical values.

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Requests for reprints should be sent to: Lorentz E. Wittmers, Jr., Department of Physiology, University of Minnesota-Duluth School of Medicine. Duluth, Minnesota 55812.

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Appendix

Calculation of Mean Skeletal Lead Concentration (Pb)

To examine skeletal lead burden, we have opted not to use or attempt to define total lead content because it is evidently a function of total skeletal mass and therefore of body build, sex, and age. It was decided that the term "mean skeletal lead concentration" (Pb) be defined and calculated:

$$(\overline{Pb})W_a = (Pb)_t W_{at} + (Pb)_s W_{as} + (Pb)_v W_{av} + (Pb)_r W_{ar} + (Pb)_i W_{ai}. \tag{1}$$

where $(Pb)_x$ is the lead concentration (in $\mu g/g$ bone ash) of the five sites, $x = t$ represents tibia, s the skull, v the vertebra, r the rib, and i the ilium. W_a represents the ashed weight of the total skeleton, and W_{ax} is the ashed weight of the five sites $(x = t, s, v, r,$ and $i)$. Unfortunately, data are not available in the literature on the ashed weight needed for the above computation. It is, therefore, necessary to estimate these values from the literature values on dry bone composition of the skeleton[25] and from our own ash to dry weight ratios (Table 1).

$$R_z = W_{az}/W_{dz}. \tag{2}$$

therefore:

$$W_{az} = R_z \times W_{dz}. \tag{3}$$

Substituting equation (3) into equation (1) and dividing both sides by the total skeletal dry weight (W_d) results in the following:

$$(\overline{Pb})W_a/W_d = (Pb)_t R_t W_{dt}/W_d \ldots\ldots\ldots + (Pb)_i R_i W_{di}/W_d. \tag{4}$$

All the values on the right side of equation (4) are known or obtainable from the literature. The W_a/W_d ratio for the total skeleton must be evaluated. This was accomplished as follows:

$$W_a = R_t W_{dt} \ldots\ldots\ldots + R_i W_{di}. \tag{5}$$

Dividing both sides by the total skeletal dry weight yields:

$$W_a/W_d = R_t W_{dt}/W_d \ldots\ldots\ldots + R_i W_{di}/W_d. \tag{6}$$

Equation (6) will produce the skeletal ash to dry weight ratio needed in equation (4) to allow calculation of mean skeletal lead concentration (Pb).

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